Utilization of common ditch vegetation in the reduction of fipronil and its sulfone metabolite

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Abstract

BACKGROUND: Fipronil, a phenylpyrazole insecticide, and its oxidative sulfone metabolite are two potential pollutants from treated rice and cotton production. A consequence of these pollutants occurring in surface runoff is degradation of downstream aquatic ecosystems. Utilization of primary intercept drainage ditches as management practices to reduce fipronil concentrations and loads has not been examined. This study used ditch mesocosms planted with monospecific stands of common emergent wetland vegetation to determine if certain plant species were more proficient in fipronil mitigation.

RESULTS: Three replicates of four plant species were compared against a non-vegetated control to determine differences in water column outflow concentrations (μg L^{-1}) and loads (μg). There were no significant differences between vegetated and control treatments in outflow concentrations ($F = 0.35$, $P = 0.836$) and loads ($F = 0.35$, $P = 0.836$). The range of fipronil reduction was 28–45% for both concentration and load. Unlike fipronil, fipronil sulfone concentrations and load increased by 96–328%.

CONCLUSION: The increase in fipronil sulfone was hypothesized as a direct consequence of oxidation of fipronil within each mesocosm. The type of ditch vegetation had no effect on fipronil reduction. Future research needs to examine initial concentrations and hydraulic retention times to examine potential changes in reduction capacities.

Keywords: fipronil; vegetation; mitigation; wetland; mesocosm

1 INTRODUCTION

Fipronil [5-amino-1-(2,6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-trifluoromethyl-sulfinylpyrazole-3-carbonitrile] is a phenylpyrazole insecticide commonly used in rice and cotton production, turf management and residential insect control (i.e. Frontline™ for ticks and fleas).[1,2] It was approved for use by the US EPA in 1996 to interfere with passage of chloride ions through GABA receptors in insects, disrupting CNS activity, resulting in hyperexcitation and death,[3,4] but as yet there are no currently mandated federal or state drinking/groundwater standards for fipronil. Fipronil, commonly sold as Regent® 4SC, is directly applied to soil furrows at the time of planting and has the potential, through runoff, to enter ditch and stream ecosystems adjacent to farms. In 1997, the US EPA stated '... based on the environmental fate assessment, fipronil and its degradates can potentially move into surface waters and are expected to exist in runoff waters primarily in the dissolved state'.[5] Differences in *Daphnia pulex* Deg. (macroinvertebrates) toxicity testing have been reported through various grades of fipronil being used. Unformulated technical-grade fipronil had no toxic effects on *D. pulex*,[6] while Regent 4SC, formulated fipronil, had both lethal and sublethal effects on *D. pulex*.[7]

Icon 6.2 FS™ is a fipronil-based seed coating approved for use in rice fields to combat rice water weevil, *Lissorhoptrus oryzophilus* Kutsch. Rice fields require large quantities of water during the growth stages, with fields being filled and drained numerous times over the growing season. Rice tailwater is often recovered and used for other agricultural purposes through post-harvest rice fields, irrigation and, in southern Louisiana, the culturing of crayfish (red swamp and white river species).[8,9] Crayfish ponds often receive direct runoff from the rice field, and are thus exposed to water potentially containing lethal concentrations of fipronil.[8] Hazard assessments have revealed that fipronil in standing water from Icon-treated rice farms poses significant risk to winter-crop crayfish survival, and potentially could have toxic downstream effects following storm events and surface runoff into receiving aquatic systems.[7]

Fipronil degrades into several products including fipronil sulfone, the major oxidative metabolite, which

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is as potent as the parent compound to invertebrates and is ninefold more potent on mammal GABA receptors.[4,10] Both fipronil and fipronil sulfone have high affinities of sediment adsorption (log $K_{ow} = 4.01$)[11,12] and potentially could remain in the aquatic environment following runoff events for extended periods of time (half-lives in sediment: fipronil 36 days; fipronil sulfone 168 days).[2] No studies thus far have evaluated the role that aquatic vegetation plays in the reduction or mitigation of fipronil and its derivatives from storm water runoff.

Drainage ditches are ubiquitous features of agricultural landscapes and are primary intercept wetlands for non-point source (NPS) pollutant runoff. Vegetated drainage ditches have been shown to mitigate NPS pollutants such as inorganic nitrogen,[13] dissolved and total phosphorus,[14] atrazine (herbicide) and lambda-cyhalothrin (insecticide),[15] chlorpyrifos,[16] metolachlor[17] and parathion-methyl.[18] For the majority, surface drainage ditches are vegetated with a variety of native and non-native obligate emergent wetland species. Geographic position and climate will play large roles in determining the species of plants that occur in any one location. In the southern USA, hot humid growing seasons and mild winters lend favorably to the establishment of a diverse group of emergent wetland plants such as *Leersia oryzoides* L. (cutgrass), *Typha latifolia* L. (cattail), *Juncus effusus* L. (common reed), *Sparganium americanum* Nutt. (bur-reed) and *Sagittaria* sp. (arrow leaf).

The aim of this study was to test the efficacy of certain species of obligate wetland vegetation in drainage ditches as buffers for fipronil and its sulfone metabolite. To the authors' knowledge, this is the first study that has examined the possible use of various species of obligate wetland vegetation in mitigating fipronil concentrations and loads as simulated by storm water runoff.

2 EXPERIMENTAL METHODS

2.1 Mesocosms, dosing and sampling

Mesocosm studies were conducted at the United States Department of Agriculture (USDA) Agricultural Research Service National Sedimentation Laboratory over the summer of 2007. Four different plant species were compared against a non-vegetated control to determine individual plant species performance in reducing fipronil concentrations in a simulated stormwater runoff. Plant species selected were *Leersia oryzoides* (L.) Sw., *Thalia dealbata* Fraser ex Roscoe, *Typha latifolia* L. and *Sparganium americanum* Nutt. All four species are native to the USA, have obligate wetland indicator status for the southeast region (region II) and are commonly observed species occurring in agricultural drainage ditches of cotton and rice.

Mesocosms were Rubbermaid™ tubs, 1.25 m long × 0.6 m wide × 0.8 m high. Mesocosms were plugged and plumbed for specific retention volumes, filled with sand as a base soil substrate and topped with approximately 7.5 cm surface layer of pond sediment collected from wetlands from the University of Mississippi Field Station. Tubs were planted with respective plant species in the summer of 2006 to ensure comparable field plant densities for the mitigation experiments in 2007. Average plant stem densities per m^2 for the respective plant species were *L. oryzoides* (1187 ± 47), *T. latifolia* (67 ± 0.3), *S. americanum* (202 ± 32.3) and *T. dealbata* (69 ± 9.8). There were three replicates for each species and the non-vegetated control (total $N = 15$).

Fipronil concentrations as high as $5.3\,\mu g\,L^{-1}$,[11] $8\,\mu g\,L^{-1}$[12] and $9\,\mu g\,L^{-1}$ [8] have been detected in surface waters downstream of rice fields planted with fipronil-treated rice seed. These values all average approximately 0.1% of a typical insecticide application rate applied to agricultural plots. A target concentration of $5\,\mu g\,L^{-1}$ was applied to each mesocosm; however, actual concentrations may have been much lower owing to adsorption to delivery tubing, mixing chambers and Rubbermaid™ mesocosm containers. Dosage of fipronil was based on a 4 h retention time within each mesocosm. The fipronil storm runoff dose was delivered via Fluid Metering Inc. (FMI™) lab pumps, models QD-1 (flow range 0–552 mL min^{-1}) and QD-2 (flow range 0–1242 mL min^{-1}), at a rate specific to each mesocosm (range 254–654 mL min^{-1}). After 4 h, clean water was delivered through new inflow and outflow delivery tubing for a subsequent 8 h period. Water samples were taken pre-exposure and every hour for 12 h from each mesocosm. Plant and sediment samples were sampled 7 days after fipronil amendment. The reason for this sampling protocol was twofold: (1) too frequent a sampling regime would have disturbed the small system and destructively removed the majority of plants; (2) a 7 day sample would provide an understanding of what proportion of pesticide (parent or sulfone metabolite) remained in the respective system compartments. Soils were sampled from the top 0–5 cm, air dried to a constant weight and ground using a Wiley Mill soil grinder. Submerged plant portions were harvested, dried to a constant weight in a greenhouse and ground with a Wiley Mill plant grinder with a 2 mm mesh diameter. Excess water from mesocosm studies was channeled to large open-air sumps, diluted 100:1 and stored for a week to ensure photodegradation of the insecticide and its metabolites.

2.2 Sample preparation and analysis

Water samples were collected in amber 1 L glass jars and were immediately fixed with potassium chloride and distilled ethyl acetate. Within 24 h, all samples were prepared for gas chromatography following the procedures outlined in Bennett *et al.*,[19] Smith and Cooper[20] and Smith *et al.*[21] All pesticide analyses (water, plant and sediment) were conducted with HP model 6890 gas chromatographs each equipped with dual HP 7683 ALS autoinjectors, dual capillary

columns and a HP Kayak XA Chemstation.[8,20,21] Table 1 highlights certain physical and chemical properties for fipronil and fipronil sulfone.[1,22,23] Analytical detection limits on the gas chromatograph for fipronil and fipronil sulfone in water and soil/plant samples were 0.01 $\mu g\,L^{-1}$ and 0.1 $\mu g\,kg^{-1}$ respectively. Individual differences between species reductions were compared with a one-way ANOVA and post-hoc Tukey honestly significantly different test (HSD; $\alpha = 0.05$) in JMP version 5.0.1.[24] Values analyzed were the natural logarithm of the concentration in the mixing chamber divided by the measured concentration in the outflow. Loads (μg) were calculated by multiplying inflow concentrations ($\mu g\,L^{-1}$) by the known inflow volume (L) from the FMI™ pumps. Outflow loads were calculated similarly, with the assumption that the inflow and outflow volumes were equal.

3 RESULTS AND DISCUSSION

3.1 Abiotic factors in fipronil reduction

Temperature and pH are important abiotic factors that control the dissipation and degradation rates of fipronil and its metabolites.[25–27] Bobe *et al*.[26] reported that variations in pH alter the rate of hydrolysis in fipronil in water and soil. The more basic the pH, the shorter the half-life of fipronil (770 h at pH 9.0, 2.4 h at pH 12.0). The hydrolysis of fipronil is stable under acid (pH 5.5) and neutral conditions. Water pH for the current study averaged 6.9 ± 0.1, with no differences occurring between plant and control treatments, suggesting a lack of hydrolysis of fipronil. Furthermore, Tingle *et al*.[27] and Bobe *et al*.[26] reported shorter half-lives for fipronil as temperature increased (22 °C, ~114 h; 30 °C, ~75 h). The present study was conducted during the summer (85 °F; 29 °C) and thus had relatively short reported half-lives for fipronil.

Table 1. Physical and chemical properties for fipronil and fipronil sulfone

Physiochemical property	Fipronil	Fipronil sulfone	Reference
Average K_{oc}	825	1447–6745	1
Log K_{ow}	3.50	4.01	1,11
Water solubility (pH 5)	1.90 mg L^{-1}	–	22
Water solubility (pH 9)	2.40 mg L^{-1}	–	22
GC retention times	27.1 min	33.4 min	
Aqueous half-life	125 h	–	23
Soil half-life	36–438 h	168 days	1,23

3.2 Concentration reduction

There were no detectable fipronil or fipronil sulfone residues within plant and control treatments prior to fipronil amendment (0 h, $n = 15$, 0 $\mu g\,L^{-1}$). There were no significant differences between initial mixing chambers for fipronil ($F = 0.96$, $P = 0.4781$) and fipronil sulfone ($F = 2.49$, $P = 0.126$) concentrations. Overall, initial fipronil and fipronil sulfone concentrations were $2.25 \pm 0.08\,\mu g\,L^{-1}$ and $0.01 \pm 0.007\,\mu g\,L^{-1}$ respectively (Table 2). Desired 5 $\mu g\,L^{-1}$ concentrations decreased as expected from the initial calculated concentrations. The temporal distribution of fipronil followed the 4 h amendment, with an increase in outflow concentration, a peak in concentration at 4 h and a tailing of concentrations throughout the rest of the experiment (Fig. 1). Unlike fipronil, fipronil sulfone, an oxidation degradate, increased throughout the experiment (Fig. 2). The sulfone metabolite formed quickly, reaching a maximum concentration approximately 10 h post-amendment (Table 2). Fipronil, under oxidation, converts to fipronil sulfone, and the latter is known to increase in concentration in biological systems.[4] Relative to fipronil, the fipronil sulfone metabolite is more persistent and more potent to many freshwater invertebrates.[4,11]

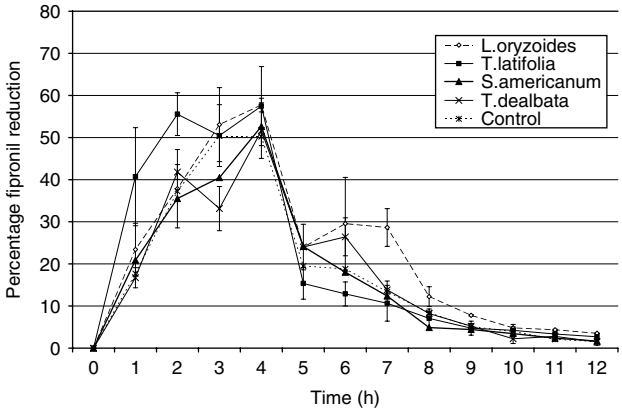

Figure 1. Mean (± SE) temporal distributions of percentage fipronil reductions in outflow for the five respective treatments. No significant differences occurred between any of the distributions ($\alpha = 0.05$).

Table 2. Temporal characteristics of fipronil and fipronil sulfone following pesticide amendment between the five treatments

Amendment characteristic	*Leersia oryzoides*	*Typha latifolia*	*Sparganium americanum*	*Thallia dealbata*	Control
Initial fipronil mixing-chamber concentration ($\mu g\,L^{-1}$)	2.10 (±0.28)	2.19 (±0.09)	2.55 (±0.04)	2.18 (±0.18)	2.29 (±0.2)
Initial fipronil sulfone mixing-chamber concentration ($\mu g\,L^{-1}$)	0.08 (±0.01)	0.08 (±0.009)	0.12 (±0.05)	0.12 (±0.007)	0.10 (±0.02)
Maximum fipronil concentration ($\mu g\,L^{-1}$)	1.20 (±0.13)	1.14 (±0.15)	1.35 (±0.25)	1.13 (±0.18)	1.4 (±0.16)
Time to fipronil peak (h)	4	4	4	4	4
Maximum fipronil sulfone concentration ($\mu g\,L^{-1}$)	0.29 (±0.06)	0.23 (±0.01)	0.27 (±0.05)	0.23 (±0.04)	0.30 (±0.01)
Time to fipronil sulfone peak (h)	10	9	10	11	9
Fipronil concentration reduction (% at 4 h)	38 (±1.9)	48 (±6.6)	47 (±9.4)	42 (±1.2)	46 (±3.9)
Fipronil sulfone concentration gain (% at 10 h)	328 (±117)	181 (±39)	129 (±34)	96 (±23)	253 (±72)

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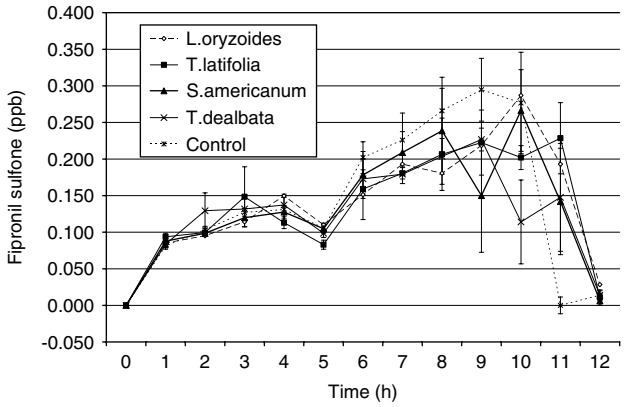

Figure 2. Mean (± SE) temporal distributions of fipronil sulfone concentrations in outflow for the five respective treatments. No significant differences occurred between any of the distributions ($\alpha = 0.05$).

There were no significant differences between vegetative species treatments and control distributions for fipronil or fipronil sulfone (Figs 1 and 2). Although there was always a 38–48% reduction from the initial concentration in fipronil over the 4 h amendment, there were no significant differences in the percentage concentration reduction between individual vegetated treatments and control ($F = 0.35$, $P = 0.836$). The order of biological reduction efficiency between the treatments was observed to be: *T. latifolia* > *L. oryzoides* > *S. americanum* > control > *T. dealbata*. This reduction could be attributed to attachment area (plant density); however, there was no relationship between fipronil reduction and plant density ($r^2 = 0.589$; $P > 0.05$). Reduction was more than likely due to adherence of pesticide to delivery tubing, mixing chambers and Rubbermaid mesocosms, as well as the conversion of fipronil to fipronil sulfone. The degradation of fipronil to fipronil sulfone, with the retention time within the mesocosm, resulted in a decrease in observed fipronil concentrations. Fipronil is known to have an average K_{oc} value of 803,[12,28] indicating moderate persistence in soil (>34 days). Soil and plant testing 7 days post-amendment reported no detectable concentrations of fipronil or its oxidative degradate in any system and soils that had very low soil organic matter contents. Connelly[28] reported that fipronil sorption was positively correlated with organic carbon content in soils.

Observed fipronil concentrations at the peak of the amendment (range 1.14–1.4 $\mu g\,L^{-1}$) would affect *Amphiascus tenuirmis* (estuarine copepod) populations. Fipronil concentrations of 0.22 $\mu g\,L^{-1}$ delayed male and female copepod growth and development, resulting in subsequent predicted population declines of 62%.[12] Fipronil sulfone concentration increased between 96 and 328% over the course of the experiment (Table 2). This increase in sulfone was hypothesized as a result of the highly oxidized system occurring within each mesocosm. Shallow water depths and constant flow maintained oxidative conditions within the water column of each mesocosm, providing a conducive environment for fipronil oxidation. Saturated soil conditions, as well as an oxygenated soil surface, will result in the formation of fipronil sulfone from the parent compound.[26,29] There are limited data available on the toxicity of fipronil sulfone, although it is stated that it is 3.3 times more toxic than the parent fipronil compound.[4,11,29] The 96 h LC_{50} values for fipronil sulfone in bluegill sunfish, rainbow trout and *Daphnia magna* were 25, 39 and 29 (48 h LC_{50}) $\mu g\,L^{-1}$ respectively, which were similar to or lower than those for fipronil.[29] Furthermore, Connelly[28] reported the sulfone degradate as being 6.3 times more toxic to rainbow trout, 3.3 times more toxic to bluegill sunfish and 6.6 times more toxic to freshwater invertebrates than the parent fipronil compound. There were no significant differences between individual wetland species and the control in increasing fipronil sulfone concentrations.

3.3 Load reduction

Tables 3 and 4 show load characteristics between treatments through time for fipronil and fipronil sulfone respectively. There was a significant difference between inflow fipronil loads, with *S. americanum*, *L. oryzoides* and the control having significantly higher loads than *T. latifolia* and *T. dealbata* (Tukey HSD; $F = 6.44$, $P = 0.01$). However, there were no significant differences between relative outflow loads for any of the treatments ($F = 2.35$, $P = 0.14$). There were no significant differences ($P > 0.05$) in fipronil load reductions between treatments for

Table 3. Fipronil load characteristics for the four vegetated and control treatments. Storm pesticide amendment occurred over the initial 4 h, and was followed by 8 h of clean water. Clean water had no detectable fipronil concentrations

Load characteristic	*Leersia oryzoides*	*Typha latifolia*	*Sparganium americanum*	*Thallia dealbata*	Control
Average loss over experiment duration ($\mu g\,L^{-1}\,h^{-1}$)	16.5 (±2.6)	12 (±0.7)	18.7 (±2)	12.5 (±1)	15.5 (±1.3)
Average loss over amendment duration (4 h) ($\mu g\,L^{-1}\,h^{-1}$)	25 (±3)	24 (±3)	32 (±7.5)	19.6 (±2.8)	26.8 (±2.4)
Relative loss in 0–4 h (%)	50 (±2)	66 (±5)	55 (±7.5)	52 (±2)	58 (±2.4)
Relative loss in 4–8 h (%)	41 (±2.4)	27 (±3)	38 (±5)	41 (±2.5)	35 (±2.1)
Relative loss in 8–12 h (%)	9 (±0.5)	7 (±2)	6.5 (±3)	6.7 (±1.2)	7 (±0.3)
Mean total outflow load (μg)	199 (±32)	145 (±8)	225 (±25)	151 (±18)	187 (±15)
Mean total inflow load (μg)	274.3 (±20)	246 (±32)	406 (±15)	268 (±33)	330 (±17)
Reduction in load (inflow - outflow) (%)	28 (±6)	40 (±5)	45 (±4)	43 (±3)	43 (±5)

Table 4. Fipronil sulfone load characteristics for the four vegetated and control treatments. Storm pesticide amendment occurred over the initial 4 h, and was followed by 8 h of clean water. Clean water had no detectable fipronil concentrations

Load characteristic	*Leersia oryzoides*	*Typha latifolia*	*Sparganium americanum*	*Thallia dealbata*	Control
Average loss over experiment duration ($\mu g\,L^{-1}\,h^{-1}$)	5 (±0.75)	4 (±0.6)	5.8 (±0.5)	4.2 (±0.7)	5.5 (±0.4)
Average loss over amendment duration (4 h) ($\mu g\,L^{-1}\,h^{-1}$)	3 (±0.5)	2.8 (±0.1)	3.7 (±0.4)	3.2 (±0.5)	3.4 (±0.2)
Relative gain in 0–4 h (%)	20.7 (±0.5)	23.5 (±4.2)	21.4 (±1)	25.5 (±1.5)	20.7 (±0.5)
Relative gain in 4–8 h (%)	34 (±3)	33 (±0.9)	39 (±3.9)	38.2 (±1.3)	40 (±2.3)
Relative gain in 8–12 h (%)	45 (±2.6)	43 (±3.3)	39 (±4.9)	36 (±2.8)	39 (±2)
Mean total outflow load (μg)	60.3 (±9)	49 (±7)	69 (±6.5)	50 (±8.2)	66 (±4.7)
Mean total inflow load (μg)	10.4 (±0.6)	13.3 (±1.5)	19.4 (±1.8)	9.9 (±1.1)	14 (±1.8)
Gain in load (inflow - outflow) (%)	476 (±55)	267 (±10)	256 (±2)	399 (±48)	401 (±109)

each time step (0–4, 4–8, 8–12) passed within the experiment. Furthermore, there were no significant differences in the percentage load reduction between the four vegetated and control treatments ($F = 1.574$, $P = 0.265$). Biologically, *S. americanum* reduced the highest amount of fipronil (45%), followed by *T. dealbata* (43%), non-vegetated control (43%), *T. latifolia* (40%) and *L. oryzoides* (28%).

Similarly, *S. americanum, T. latifolia* and control fipronil sulfone inflow loads were significantly higher than those of *L. oryzoides* and *T. dealbata* (Table 3). Likewise, when outflow loads and percentage gained were compared between treatments, there were no significant differences ($F = 1.5$, $P = 0.28$; $F = 1.802$, $P = 0.2205$) (Table 3). The percentage load reductions (Table 2) for fipronil were slightly lower than the concentration reductions (Table 1), with a range of 28–45%. Again, the absence of significant differences between load reductions suggests that the biogeochemical process common to all mesocosms could be the reduction process, rather than individual species of plants and their plant density.

3.4 Fate of fipronil and sulfone metabolite

The decrease in fipronil and its sulfone metabolite concentrations and loads in these mesocosms was hypothesized as a result of three processes: oxidation, photolysis and biological degradation. The present study showed a 28–48% decrease in fipronil within the mesocosms. Fipronil degradation under anaerobic conditions is extremely slow ($t_{1/2} = 116$–130 days), and thus reduced and anaerobic conditions for the systems were ruled out. Ngim and Crosby[23] demonstrated that the dissipation of fipronil in water can be explained by a combination of processes strongly favored towards photolysis. UV irradiance and photolysis is likely to have resulted in the degradation of fipronil in the present study. However, the greater persistence of the sulfone metabolite in all mesocosms seems to suggest a strongly oxidative environment. The flow of water, a shallow water depth (>15 cm), dense stands of metabolizing vegetation and algae and high temperatures were all conducive to oxidation of fipronil. Biological degradation of fipronil by bacteroplankton and plankton in suspension, as well as algae attached to submerged plant surfaces, was more than likely secondary to creating an oxidizing hydrosphere for fipronil degradation.

4 CONCLUSION

Fipronil, a GABA system blocker insecticide, is commonly used in rice and cotton agriculture, and thus is prone to surface runoff into aquatic environments. There were no differences in fipronil and fipronil sulfone outflow loads or concentrations within wetland ditch mesocosms using a variety of obligate wetland plants and a non-vegetated control. Similar wetland biogeochemical conditions within each mesocosm resulted in consistent 38–48% reductions in fipronil, yet yielded a significant increase in the sulfone metabolite through time. This increase is hypothesized to be derived from the highly oxidized vegetated system. Fipronil reduction was a function of unavoidable adherence to the delivery system, mixing chamber and mesocosms as well as the rapid oxidation to fipronil sulfone. Will a larger residence time improve the reduction capacity of drainage ditches in fipronil mitigation? Would a longer residence time reduce toxic fipronil degradate concentrations below lethal limits? Future research on variable hydraulic retention times and initial inflow concentrations will highlight changes in reduction percentages through drainage ditch mesocosms.

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